

EFFECTS OF LIGHT AND AGING ON RIBONUCLEOTIDE COMPOSITION IN *AVENA SATIVA*^{1, 2}

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ABSTRACT

This investigation was carried out to study the effects of light on the ribonucleotide-base composition of coleoptiles and primary leaves of oat seedlings (*Avena sativa* L., var. *victory*). The effects of aging on the base composition of primary leaves were also studied. The nucleotides obtained by alkaline hydrolysis of ribonucleic acid were separated by eluting a Dowex-1-X8 column with formic acid and ammonium formate.

Aging from 5 to 15 days was accompanied by a significant decrease in guanosine monophosphate content in both light-grown and dark-grown primary leaves. Light treatments appeared to have a counteracting influence in the effects of aging on guanosine monophosphate content. These observations are consistent with the hypothesis that ribosome breakdown with age can partially be inhibited by light.

INTRODUCTION

Analyses of ribonucleic acid from various plant organs have not been conclusive in the demonstration of differences in nucleotide-base composition between respective RNA fractions. Ross (1962) has found the nucleotide composition of total RNA from vegetative buds and immature flowers of *Xanthium* shoot tips to be similar. However, Yoshida *et al.* (1967) have reported significantly reduced levels of guanosine monophosphate and cytidine monophosphate in photoperiodically induced cotyledons of *Pharbitis nil* as compared with those of noninduced cotyledons. RNA extracted from one- and two-dimensional plantlets of *Dryopteris* (Hotta, Osawa, and Sakaki, 1959) have different base ratios [one-dimensional, cytidine monophosphate(CMP)/adenosine monophosphate(AMP)/uridine monophosphate(UMP)/guanosine monophosphate(GMP) = 5.8/8.2/10.0/11.0; and two-dimensional, 11.4/10.9/10.0/14.0]. Recently, however, Howland (1972) has reported these differences to be due to an increase in the proportion of chloroplast ribosomal RNA (r-RNA) and not to the initiation of prothallial growth. A study of r-RNA nucleotide-sequence homologies by means of competitive nucleic acid hybridization experiments has shown no major differences between root and shoot fractions of pea seedlings (Trewavas and Gibson, 1968).

One of the most widely accepted parameters of leaf senescence is the decline of RNA content (Addicott, 1969). Although few studies have been carried out on the effects of aging on nucleotide-base composition in plants, centrifugation and electron microscope work indicate that ribosomes are unstable in senescing cells. Eilam, Butler, and Simon (1971) have found that the content of both free and bound ribosome in aging cucumber leaves increases until just before maximum leaf size is reached and then decreases during senescence.

Relatively little is known concerning the effects of light on the nucleotide composition of various plant-RNA fractions. Light apparently enhances chloroplast r-RNA synthesis in cucumber, pea, and barley leaves, but such synthesis still occurs in the dark, although at a reduced rate (Harel and Bogorad, 1973; Scott, Nair, and Smillie, 1971; Smith, Stewart, and Berry, 1970). Ribosomal RNA from both etiolated and from green *Euglena* cells has AMP/GMP/CMP/UMP/compositions of 10/13.3/10.5/9.77 and 10/11.8/11.1/9.5, respectively (Brawerman and Chargaff, 1959). In this case, illumination is accompanied by an increase in the AMP to GMP ratio. However, the extrapolation of inferences from these data

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to higher plants is somewhat tenuous because of known differences between ribosomes of *Euglena* and those of higher plants.

Information on changes in nucleotide composition of RNA of various plant organs at different ages, as well as the effects of environmental interactions, would be of considerable value in elucidating control mechanisms of developmental responses. In the present study, the effects of light and aging on the nucleotide composition of RNA of primary leaves of *Avena sativa* and the effects of light on that of coleoptiles were investigated.

MATERIALS AND METHODS

Oat seeds (*Avena sativa* L., var. *victory*) germinated for four days in darkness at 28°C in sterilized vermiculite. Following germination, growth was permitted to continue under relatively constant conditions of temperature and humidity either in the dark or in continuous light. No nutrients were applied to the seedlings during the course of the experiment. The only source of nutrients or food reserves were those present in the seed.

RNA was extracted from the following organs: (a) light- and dark-grown primary leaves of 5-day-, 10-day-, and 15-day-old oat seedlings; and (b) light- and dark-grown 5-day-old coleoptiles. RNA extracts were prepared by the sodium dodecyl [sulfate phenol cetyltrimethylammonium bromide] extraction method (Ralph and Bellamy, 1964). The purity of these preparations was determined by their ultraviolet absorption spectra at 240–300 nm, as measured with a Beckman DU spectrophotometer.

RNA preparations from coleoptiles and primary leaves were hydrolyzed with 0.3M KOH and incubated at 37°C for 16 to 18 hours (Bock, 1967). The hydrolysates obtained were chilled and neutralized with perchloric acid. After the insoluble KClO_4 was removed by centrifugation, the pH of the supernatant liquid was adjusted to 10 with 3M KOH. After chilling on ice for 10 minutes, the suspension was centrifuged and the resulting liquid was stored in a freezer for subsequent column chromatography.

Dowex-1-X8 (200 to 400 mesh) was used as an anion-exchange resin. CMP, AMP, UMP, and GMP were eluted with 0.025N HCOOH , 0.20N HCOOH , 0.015N HCOOH –0.20M ammonium formate, and 0.10N HCOOH –0.20M ammonium formate, respectively, and were collected in 5-ml fractions (usually 9 to 15 fractions for each nucleotide). The flow rate was maintained at 1 ml per minute. The peaks were identified by their characteristic 280/260 ratios as follows: CMP, 1.80; AMP, 0.23; UMP, 0.28; and GMP, 0.68. The optical density for each fraction was determined at 280 nm for CMP and at 260 nm for AMP, UMP, and GMP.

Total optical density readings for all fractions of each nucleotide were calculated by the following method: the number of moles of a particular nucleotide was determined by dividing the total optical density by the appropriate extinction coefficient (A_m) value (Chargaff and Davidson, 1955; Pabst Brewing Company, 1965).

The A_m values for the four nucleotides are as follows: CMP, 13.0×10^3 ; AMP, 14.7×10^3 ; UMP, 10.0×10^3 ; and GMP, 11.7×10^3 . The molar percentages of the respective nucleotides were determined by dividing the number of moles of the individual nucleotide by the total number of moles of the four nucleotides.

RESULTS

In light- and dark-grown primary leaves, μg of RNA/g fresh weight of tissue decreased from day 5 (5 days after planting) to day 15 (fig. 1A). The RNA content/green leaf of plants which had been exposed to light for one day (*i.e.*, a 5-day-old plant) was about the same as that of the etiolated leaf (fig. 1B). However, the RNA content in coleoptiles was less than 20 percent of that in the primary leaves on the basis of μg RNA/g fresh weight of tissue (fig. 1A).

Ribonucleic acids from 5-day-old coleoptiles and 5-day-old primary leaves were both GMP-rich (Table 1). In 5-day-old coleoptiles, the UMP content was lower and the AMP content was higher than in 5-day-old primary leaves grown either in dark or in light. RNA from 10-day-old green and etiolated leaves were both GMP-rich, the GMP-to-AMP ratios of both green and etiolated leaves being similar at this age. Light treatment was accompanied by a decrease in UMP in both coleoptiles and primary leaves at all ages for which measurements were made (Table 1). The effect was significant for 5- and 10-day-old primary leaves. The 15-day-old etiolated leaves showed signs of premature senescence.

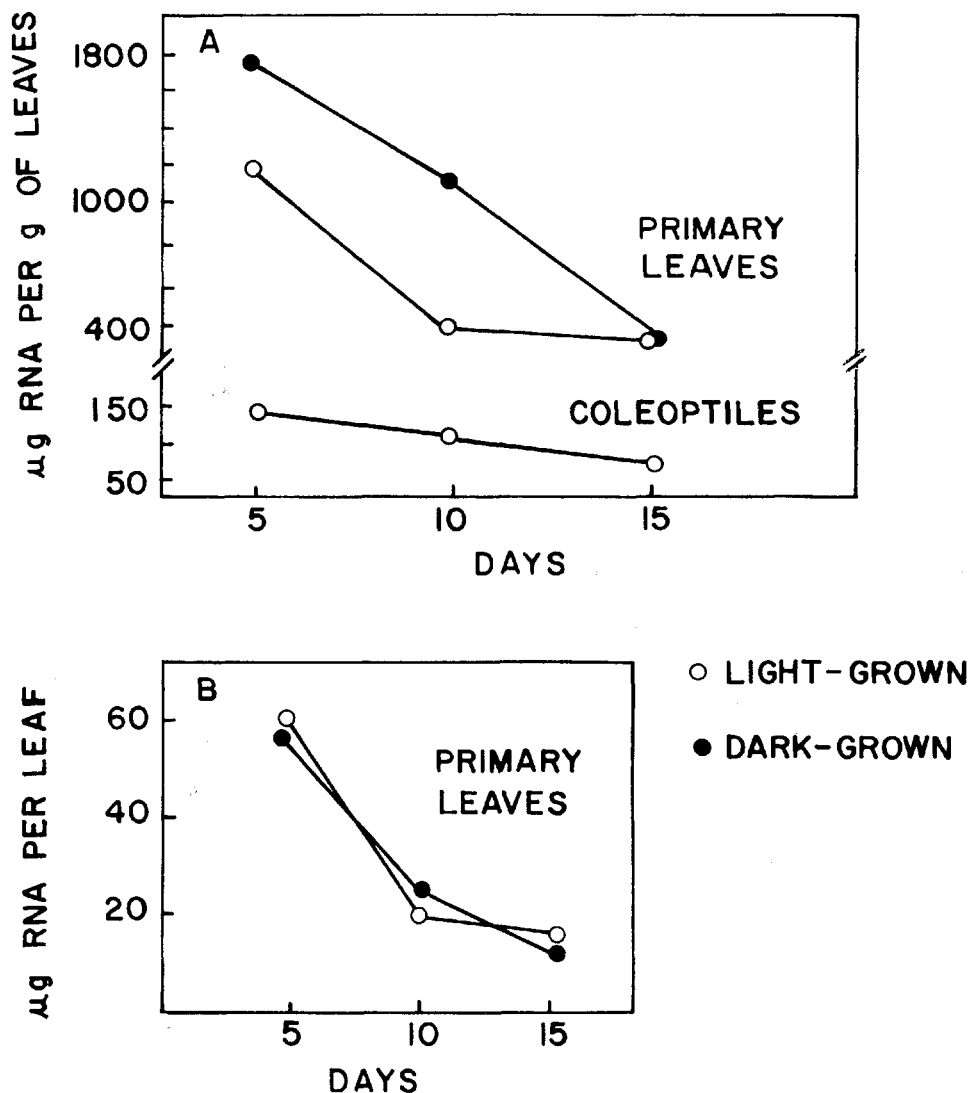


FIGURE 1. A, Changes in total RNA content/g fresh weight of coleoptiles and of primary leaves with age in light and dark. B, Changes in total RNA content/primary leaf with age in light and dark. Light-grown seedlings were illuminated beginning from day 4. Each point represents the average of two or three experiments.

This was probably due to the fact that the seedlings had exhausted their source of food reserves from the seeds. The RNA of both green and etiolated 15-day-old leaves was low in GMP.

The major change in the nucleotide composition with age occurred in GMP content. There was a very significant decrease in GMP in primary leaves with increasing age under both light and dark conditions (Table 1). This was particularly evident in the extremely low GMP-to-AMP ratio of the 15-day-old dark-grown seedlings. The primary leaves in light were always accompanied by a higher GMP level than were those grown in dark, especially at the age of 15 days.

TABLE 1

The nucleotide composition of RNA from light- and dark-grown coleoptiles and primary leaves at various ages. Each value represents the average of three experiments. $P=0.05$

Organ (condition)	Age in days	Nucleotides (molar percentage)						
		GMP	AMP	UMP	GMP	GMP/AMP	$\frac{\text{AMP}+\text{GMP}}{\text{CMP}+\text{UMP}}$	AMP/UMP
coleoptile (dark)	5	22.1 \pm 4.81	27.4 \pm 3.23	16.7 \pm 5.16	33.8 \pm 4.02	1.23	1.58	1.64
coleoptile (light)	5	25.7 \pm 0.35	30.7 \pm 0.35	12.5 \pm 0.35	31.1 \pm 0.35	1.01	1.62	2.46
primary leaf (dark)	5	24.2 \pm 0.00	23.9 \pm 0.25	22.5 \pm 0.35	29.4 \pm 0.00	1.23	1.14	1.06
primary leaf (light)	5	23.9 \pm 0.35	24.3 \pm 2.37	17.9 \pm 2.14	33.9 \pm 1.02	1.39	1.39	1.36
primary leaf (dark)	10	22.1 \pm 0.54	26.4 \pm 0.54	24.7 \pm 1.02	26.8 \pm 0.00	1.02	1.14	1.07
primary leaf (light)	10	24.9 \pm 0.35	27.0 \pm 0.69	19.9 \pm 0.35	28.2 \pm 0.00	1.04	1.23	1.36
primary leaf (dark)	15	33.7 \pm 2.32	35.1 \pm 1.36	21.6 \pm 2.08	9.6 \pm 2.50	0.28	0.81	1.62
primary leaf (light)	15	32.9 \pm 0.35	25.2 \pm 0.00	20.3 \pm 0.00	21.6 \pm 0.35	0.86	0.88	1.24

DISCUSSION

The fact that the RNA fraction of coleoptile tissue contained less than 20 percent of the amount of RNA found in primary leaves on an equivalent fresh-weight basis was puzzling. One obvious answer might be that only one cell layer in the five or six cell layers of a coleoptile contains chloroplasts. On this basis most of the excess RNA present in the primary leaves probably would be chloroplast r-RNA. However, polyacrylamide-gel-electrophoresis data (Cline, M. G., unpublished; Loening and Ingle, 1967) indicate that the bulk of r-RNA in very young leaves is cytoplasmic (25s and 18s) rather than chloroplast (23s and 16s). Therefore a more complete explanation is that the small unexpanded primary leaves contain many more cells (and, hence, more RNA) per gram of tissue than do the 5-day-old coleoptiles with their elongated cells. On the other hand, in older, expanded green leaves, the chloroplast r-RNA probably would contribute substantially to the total r-RNA (Smith, Stewart, and Berry, 1970).

There were some differences between the nucleotide composition of RNA in 5-day-old coleoptiles and that in 5-day-old primary leaves. Since the whole RNA extracted in these experiments consisted mainly of r-RNA and, to a lesser extent, of transfer RNA and of other possible kinds of RNA (including RNA degradation products), these differences probably reflect the relative proportions of r-RNA to other kinds of RNA. The GMP-to-AMP ratios were equal in 5-day-old dark grown coleoptiles and in etiolated primary leaves, whereas the AMP-to-UMP

ratios were comparatively much higher in coleoptiles. The significance of this is not known.

Tester and Dure (1967) reported base ratios of etiolated oat coleoptiles and primary leaves after a 3-day germination period. These authors' data (CMP/AMP/UMP/GMP=23.7/22.4/23.1/30.8) are similar to data reported here for the nucleotide composition of 5-day-old etiolated primary leaves. This is not surprising, because a coleoptile segment with enclosed primary leaves contains most of its RNA in the primary leaves, so that base ratios primarily reflect those of RNA from the primary leaves. Their AMP+GMP-to-CMP+UMP ratio (1.14) was exactly the same as was that of our 5-day-old etiolated primary leaves (Table 1).

One of the most significant results of these experiments was the marked decrease of GMP with age in both the light- and dark-grown primary leaves. Since r-RNA is known to be GMP-rich and is known to constitute the bulk of total RNA, it follows that this GMP decrease in total RNA is suggestive of a decline in the proportion of r-RNA in the total RNA. This decrease in r-RNA may have been due to r-RNA breakdown during ribosome decay in the senescing tissue. Eilam *et al.* (1971) have suggested that decreases in ribosome content may be due to an accelerated breakdown of existing ribosomes, or to a slowing down of ribosome synthesis, or both. Transfer RNA (t-RNA) is also high in GMP content, but since t-RNA constitutes such a small portion of the total RNA, it is highly doubtful that a decrease in t-RNA could account for the substantial reduction of GMP observed in the total RNA.

Wollgiehn (1967) has found r-RNA of excised tobacco (*Nicotiana rustica*) leaves to be very labile and to decrease more rapidly than soluble RNA or DNA during aging. Srivastava (1967) found no decrease in GMP in excised barley leaves that were floating in water for 4 days in the dark at 22–24°C. However, it is difficult to make any significant comparisons of data, because of the many differences in experimental conditions between Srivastava's investigations and those of the present study, especially in regard to the aging periods.

A highly interesting aspect of this GMP decrease with age is the effect of the light treatment. The decrease of GMP with age was much greater in the dark-treated leaves than in the light-treated leaves. Hence, light appears to have had some kind of inhibiting effect on the age-dependent decrease in ribosome content. This could be accomplished by the stabilization of the ribosome structure or by the promotion of ribosome synthesis. The production of high-energy compounds as a result of photosynthesis in the green leaves could certainly aid in such stabilization and/or promotion. It is also of interest that light treatments were accompanied by some decreases in UMP in each organ at all ages tested. It should be emphasized that these seedlings were grown on vermiculite without fertilization and therefore the only sources of nutrition were those food reserves in the seeds, and in the applied water. It is possible, in the case of the 15-day-old seedlings, that mineral deprivation, as well as aging, played a role in the reduction of GMP.

Smith *et al.* (1970) point out that, in dark-grown barley seedlings (*Hordeum vulgare*, cv. Procter), a 24-hr light treatment increases chloroplast r-RNA levels markedly in young leaves (2- to 4-day-old seedlings), but has a progressively smaller effect on older leaves (5- to 7-day-old seedlings). By the sixth day, chloroplast r-RNA accumulates to about 50 percent of the total r-RNA.

It appears that in the senescing, the green, and the etiolated leaves there was a substantial breakdown in ribosomes and a significant decay of r-RNA. These observations are also in accord with the conclusions drawn by Loening (1965), who suggests that the effect of excising root segments from the whole root is similar to "step-down" conditions in bacteria, inasmuch as r-RNA synthesis is inhibited, and by Richmond and Biale (1967), who point out that degeneracy in avocado cells is accompanied by a decrease in r-RNA synthesis.

Exogenous applications of abscisic acid (ABA) are known to accelerate senes-

cence in leaves (Addicott and Lyon, 1969; Wareing and Ryback, 1970). In addition, there is evidence that in mineral-deprived and in aged tissues, levels of endogenous ABA increase (Mizrahi and Richmond, 1972; Mayak, Halevy, and Katz, 1972). It is also well established that ABA decreases RNA content (especially r-RNA) in many tissues. Trewavas (1968) has suggested that, since r-RNA synthesis is so sensitive to the presence of various hormones, and since the level of r-RNA in the cell is usually the rate-limiting step for polysome formation, hormones could control protein synthesis by their control over the level of r-RNA. Hence, it is possible that hormones (including ABA) could control aging through their effect on r-RNA. On this basis, the marked decline in GMP which accompanies aging in primary oat leaves could be due to an endogenous ABA-caused decrease in r-RNA. Whether ABA might decrease r-RNA levels by a direct inhibition of transcriptional processes, or by promoting ribonuclease synthesis, or by some other mechanism, is not known.

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Human Osteology: a Laboratory and Field Manual of the Human Skeleton. William M. Bass. Missouri Archaeological Society. University of Missouri, Columbia, Missouri. 1971. 270 p. \$6.00.

Human Osteology represents a needed contribution to the literature of Physical Anthropology and Human Anatomy. It serves as an excellent introductory text on the anatomy of the human skeleton, bridging the literary gap between the wealth of detail contained in medical texts and the lack of detail contained in most popular accounts. Further, the work serves to update many of the older manuals on physical anthropology without the presentation of masses of detail.

The text has been designed for the beginning student. It is a tribute to Bass that the manual may be used with or without reference to skeletal specimens. The volume describes the basic anatomy of each bone, and clearly delineates the major anatomical landmarks. Individual sections of complex bones are also considered in detail. Each bone is illustrated in several views and in many cases views of the same bone from individuals of various ages are illustrated side by side for comparative purposes. It would have been desirable if some of the illustrations could have been on a larger scale; however, the illustrations are adequate for their purpose. The work is highly unusual in that criteria for differentiating between the right and left member of paired bones are given in all cases.

The consideration of human dentition, highly significant to the archaeologist and physical anthropologist, is of the highest caliber. The identifying criteria for the individual tooth types are spelled out in full, and the anatomy necessary to differentiate the individual teeth within a group, and to differentiate maxillary from mandibular teeth are well presented. The consideration of cusp-pattern development and occlusal attrition do not measure up to the remainder of the section on dentition.

The work has been organized into a number of sections based on the individual bones and bone groups of the body. This organizational method has resulted in exceptional clarity in the presentation of the osteological data. However, the author has also attempted to present information on the determination of sex, the determination of age at death, and the reconstruction of stature for many of the individual bones. This has unfortunately resulted in the absence of any theoretical consideration of age and sex determination and the reconstruction of stature.

The text is accompanied by three appendices that will be of great value to the beginning student. Appendix 1 contains a glossary of anatomical terminology and a classification of bones by shape. Appendix 2 provides a word analysis and a listing of plurals for the names of the bones and the anatomical features. Appendix 3 considers the excavation and treatment of skeletal remains from archaeological sites and forms a welcome addition to such a volume. The bibliography is very up to date, and Bass has avoided listing sources from obscure and unobtainable journals. As such, the bibliography will be of great assistance to those who wish to investigate specific topics in greater depth.

Human Osteology is a refreshing and needed addition to the literature of Physical Anthropology and Human Anatomy. It is highly recommended for the beginning student of the human skeleton and for those who wish to refresh and update their background.

JOHN EDWARD BLANK